IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| In re Application of: | ) | Art Unit: 1643 |
| CLASSEN, John B. | ) | Examiner: BRUMBACK, B. |
| Serial No.: 08/591,651 | ) | Washington, D.C. |
| Filed: February 12, 1996 | ) | September 7, 1999 |
| For: METHOD AND COMPOSITION FOR AN EARLY VACCINE... | ) | Docket No.: CLASSEN=1A |

**DECLARATION OF J. BARTHELOW CLASSEN**

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

I hereby declare:

1. I am the inventor of the invention disclosed in the above application and the President of Classen Immunotherapies.

2. I have conducted experiments which show that early immunization with a viral immunogen, a hepatitis B vaccine, reduces the rate of incidence of diabetes later in life.

Considered 9-23-99

A group of 20 female NOD mice received hepatitis B vaccine diluted in saline to a concentration of 1 part vaccine, 20 parts saline. Mice were injected intraperitoneally with 0.1 ml of vaccine on day 3 of life and intramuscularly with 0.1 ml on day 28 of life. Mice were followed for the development of diabetes. There were two controls. One group (24 female NOD mice) received no injections at all. A second group, of 20 female NOD mice, received intraperitoneal injections of saline on day 8 (.1ml), day 15 (.15ml), day 29 (.2 ml) of life. Control mice were followed for the development of diabetes.

The results are shown in the attached figure. The rate of diabetes in the group receiving the hepatitis B vaccine starting at birth were significantly lower than the rates in the control groups. The rates in the control groups were comparable and the lines intersected at several places.

*I hereby declare that all statements made herein of my own*

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*knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.*

J. Barthelow Classen

9/6/99
Date

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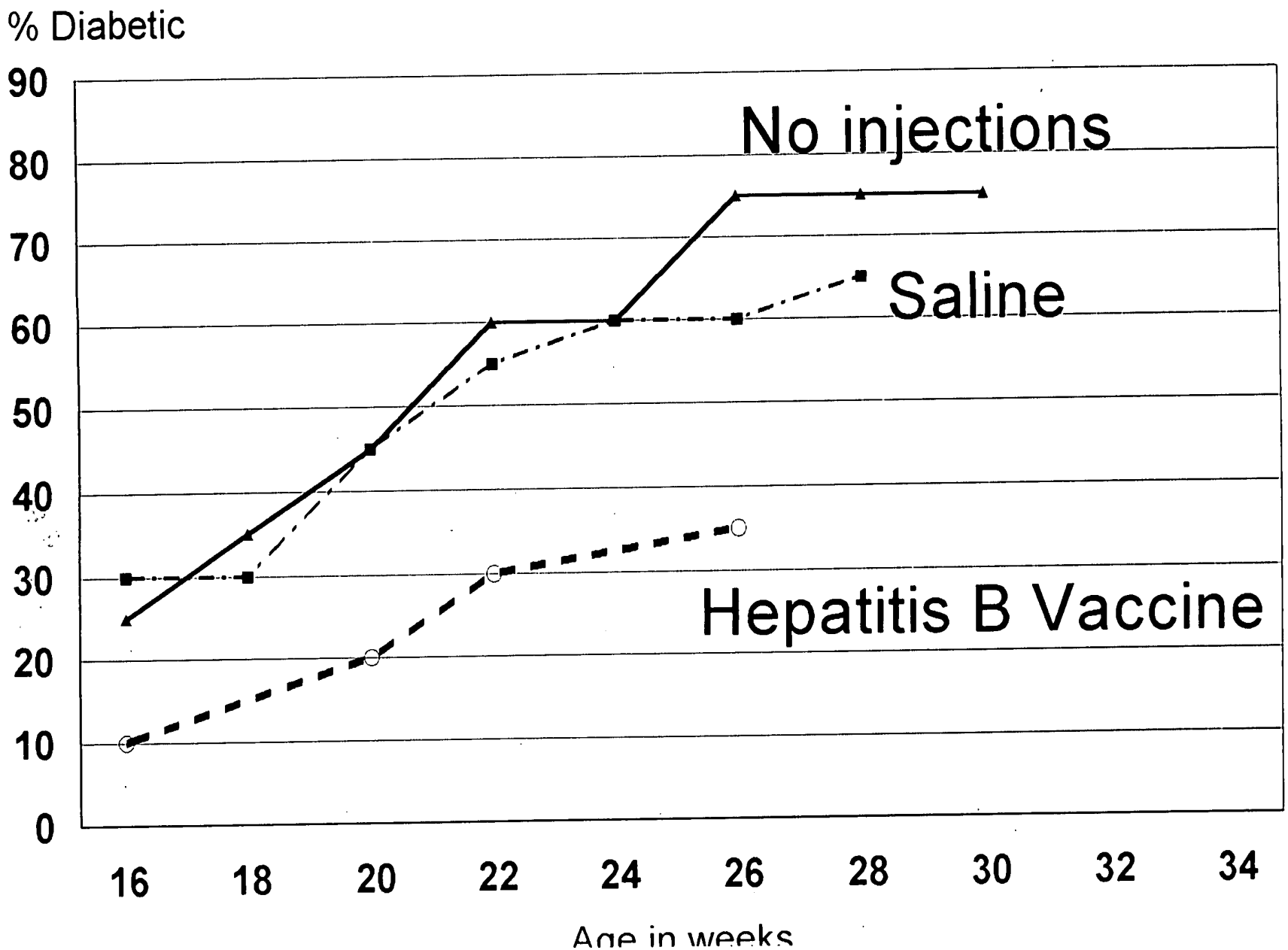
Date: 9/2/99 Time: 8:47:06 PM
From: Bart Classen To: Mr. Iver Cooper

# Hepatitis B vaccine at birth prevents diabetes in mice



Reference to Vertical transmission induced diabtes

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OBSERVATIONS

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Letters

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# Enteroviral RNA and IgM Antibodies in Early Pregnancy and Risk for Childhood-Onset IDDM in Offspring

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The prevalence of diabetes is very high in individuals with the rubella embryopathy syndrome, and recent studies have shown increased levels of enteroviral, and specifically coxsackie IgM, antibodies during pregnancy among mothers whose children later developed diabetes compared with that found in control subjects (1–3). We have now analyzed the prevalence of enteroviral RNA and IgM antibodies in the first trimester of control mothers and of mothers whose children became diabetic. We also analyzed antibodies to glutamic acid decarboxylase (GAD), a [Image]-cell antigen that has been shown to share an amino acid sequence with one of a coxsackie virus protein (4). Serum samples collected routinely during the first trimester of pregnancies in 85 mothers whose children developed diabetes before the age of 15 as recorded in the Swedish Childhood diabetes register (5) were compared with sera from 172 mothers whose children had not developed diabetes. Control subjects were selected at random in strata by county and year of birth. The distribution of month of birth was similar for both groups of subjects. Enterovirus RNA was analyzed using a nested polymerase chain reaction (PCR) (6). Enterovirus IgA, IgM, and IgG titers were analyzed using conventional enzyme-linked immunosorbent assay (7) and GAD antibodies by radioimmunoassay (8).

Three mothers of diabetic patients and one mother of control subjects were RNA positive. In addition, mothers of three patients had IgM antibodies to coxsackie B virus, but no control mother was positive. Thus 6 of 85 mothers of diabetic children had signs of an ongoing or recent enterovirus infection in early pregnancy compared with 1 of 172 control mothers, equal to an odds ratio of 12.9 (95% CI 2.43–69.52). IgG and IgA antibody concentrations did not differ between the groups. The distribution of birth months was similar among the two groups. The mean age at onset of diabetes was 7.01 ± 3.5 years among all patients, and the age at onset of patients with possible intrauterine infection was not significantly different (6.5 ± 3.0 years). The mean GAD index was higher among mothers of diabetic patients compared with mothers of control subjects (P = 0.03), but when setting the cutoff for GAD positivity at 99% of control subjects, there was no difference in positivity rate between mothers of diabetic patients and control subjects. None of the PCR+ mothers were GAD+ (Table 1).

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This latter finding does not rule out the possibility of antigen mimicry, but there are other possible mechanisms to explain an association between fetal virus infection and later development of type 1 diabetes. One could be that the perinatal infection leads to a persistent low-grade infection that might slowly hit the [Image]-cell directly and/or lead to peri-insular infection. In the latter case, the resulting inflammation could then act on the [Image]-cell according to the so-called "innocent bystander" theory.

To our knowledge, for the first time, enterovirus RNA has been detected early in pregnancy in mothers of children who later become diabetic in a higher frequency than that found in mothers of control subjects. The numbers are low and therefore need confirmation, preferably by large prospective cohort studies, where sera could be analyzed immediately to ensure that RNA is not destroyed during long-term storage. Taken together with the higher frequency of coxsackie IgM positivity reported in this study and others (2,3), and with the previous demonstration of fetal rubella infection as a strong risk factor for type 1 diabetes, evidence is accumulating that fetal viral infections might be etiologically associated with childhood type 1 diabetes, introducing new possibilities for primary prevention.

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